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Report Title

Genetically Programming Interfaces between Active Materials, Conductive Pathway and Current Collector in Li-Ion Batteries

ABSTRACT

See attached.

Genetically Programming Interfaces between Active Materials, Conductive Pathway and Current Collector in Li Ion Batteries

Kang Xu ^{a *}, Dahyun Oh ^b, Hyunjung Yi ^b, Jifa Qi ^b, Alice Xu ^{a **}, James Snyder ^c, and Angela M. Belcher ^{b, d, e}

^a Sensor and Electron Devices Directorate,
U. S. Army Research Laboratory, Adelphi, MD 20783

^b Department of Materials Science and Engineering,
Massachusetts Institute of Technology, Cambridge, MA

^c Weapon Materials Research Directorate,
U. S. Army Research Laboratory, Aberdeen Proving Ground, MD 21001

^d The David H. Koch Institute for Integrative Cancer Research,
Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

^e Department of Biological Engineering,
Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

* Corresponding author

** Summer intern apprentice under SEAP program

In this work we genetically programmed the M13 virus, so that the new clone expressed multifunctional coat protein sequences that selectively bind to designated surfaces and species. Using these new clones, composite network consisting of ironphosphate nanowire (a-FePO₄) and single wall carbon nanotubes were self-assembled onto aluminum surfaces. Spectroscopic and electrochemical characterizations confirmed the 3D-structure as well as the electrochemical activity of such composite nanowires.

Introduction

While the researchers pursue nano-structured materials for the benefits in rate of energy storage or efficiency in power conversion, several pioneering efforts were made to leverage the inspirations from the Nature. Already was the elegant 3D structure of diatom used as a template to construct SiO₂ or Si based materials, ¹ while tobacco mosaic viruses (TMV) provides scaffold for various battery chemistries ². More prominent was the series of work performed by Belcher et al, who used M13 bacteriophage as a nanometric template to biomineralize a wide spectrum of interested materials into nano-structures, with applications ranging from semi-conductor, catalyst, to Li-ion battery chemistries including Co₃O₄ as anode to a-FePO₄ and metal fluorides as cathodes. ³⁻⁵ In particular, the cycle life as well as rate capability of a-FePO₄ are comparable to the best of the nanophosphate prepared through conventional approaches. ⁵

Compared to other bio-templates used, the advantages of M13 phages exist not only in their well-understood genotype-phenotype relation, so that their corresponding genes could be altered relatively easily in order to introduce desired binding sites, but also in the fact that those phages can be produced at high efficiency in vitro, instead of

being collected and enriched from diluted ambient of the living systems. The latter is of particular significance to the feasibility of such materials, considering that a typical 18650 Li ion cells consumes at least 10 g electrode materials, therefore the annual productivity of materials to satisfy the demand for consumer electronics already amounts to thousand tons, not to mention their potential applications in a much larger market of electrified vehicles.⁷

With 4~5 nm in diameter and ~800 nm in length, M13 phage has a perfect physical shape to serve as template for nanowire (Figure 1).⁸ Its major coat proteins consist of ~2700 copies of short peptide (ca. 50-mer) called pVIII, which encase a circular single strand DNA of ~7200 base pairs, while one of the minor proteins, 5 copies of pIII that are usually 406-mer peptides, are located and exposed at one of the termini. The sections in the DNA that encode pVIII and pIII, called gVIII and gIII respectively, were well-understood and flexible to engineering.

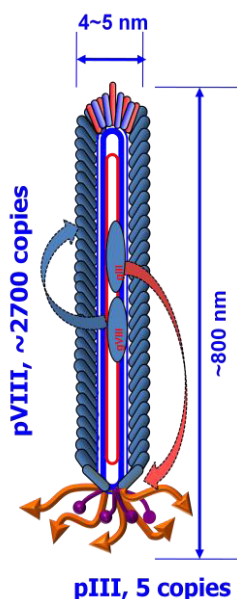


Fig. 1. Schematic illustration of M13 virus structure.

In this work, we leveraged the capability of M13 phages in bio-mineralizing interested electrochemically active particles and explored the feasibility of employing these viral templates to self-assemble mono-layers of battery cathode materials onto the most common current-collector surface. This would have potential benefit of increasing the intimate contacts for faster electron transfer during electrochemical reactions.

Experimental

Commercial phage display libraries Ph. D.-7 from NEB was used for identification of protein sequences that bind to aluminum surface.⁸ The repeated binding/elution procedure, known as “bio-panning”, was conducted according to the NEB Manual for 4~5 rounds until consensus sequences were identified by genetic sequencing. M13 phage clones that have been previously engineered by Belcher’s group to express either negatively-charged tetraglutamate (E4)⁴ or single-wall carbon nanotube (SWNT)-binding DYESALP (EFE)⁶ at pVIII were used as sources of vectors for creating new clones of multifunctional M13 phages. At gIII of their circular DNA, cutting sites were already introduced for restrictive enzymes *EagI* and *Acc65I*. Typically, vectors were

extracted and from these phages and then purified. After digested with restrictive enzymes, the double-cut genes of ~ 7000 bp were separated by agarose-gel electrophoresis from uncut DNA pieces, followed by desalting and dephosphorylation. Finally, custom-synthesized oligonucleotides (Integrated DNA Technologies, Inc.) that encode the desired peptide sequences, either identified by “bio-panning” or by “rational design”, were fused with the double-cut vector using T4 DNA-ligase, producing a new circular DNA. These recombinant DNAs were then injected into XL electro-competent blue cell at by electroporation. The new phage clones were thus collected and purified. DNA sequencing was performed to confirm that the desired pIII sequences were indeed expressed onto the new clones, while the original E4 or EFE pVIII expressions were maintained.

Binding tests were carried out by incubating aluminum foil or sapphire in given number of viral particles for 2 hours, followed by washing with TBS buffer and then elution by glycerin-HCl (pH=3.3).

Bio-mineralization of α -FePO₄ was carried out by following the mature protocol established by Belcher's group.³⁻⁶ To confirm that the new pIII expression does not interfere with the chemical composition of the active particles crystallized on the pVIII sites, nanowires of α -FePO₄ were grown using these new clones as templates, and transmission electron microscope (JEOL 200CX TEM) along with chemical analysis using EDX (JEOL 2010 HRTEM) were conducted. Additionally, ESEM/SEM (Philips XL30) and AFM (Veeco) were also used to visualize the assembly of M13 phage clones on aluminum.

Finally, with the purpose of generating the electrochemically active monolayers of α -FePO₄ on current collector, in-situ bio-mineralization of α -FePO₄ onto the M13 phage clones that were pre-immobilized onto aluminum surface was attempted by adapting a protocol reported by Lee et al.⁵ Typically an aluminum disc of 1.27 cm² in area was incubated in concentrated (2×10^{12} particles) phage suspension in TBS for 2 hours, then it was subsequently exposed to SWNT dispersion in sodium cholate, solutions of silver acetate (AgOAc), iron chloride (FeCl₃) and trisodium phosphate (Na₃PO₄), respectively. Incubation in each solution lasted for 2 hours and were both preceded and followed by repeated rinsing with TBS buffer. The final electrodes were dried at 120 °C overnight under vacuum and then assembled into coin cell with metallic lithium as counter electrode. Electrochemical characterization was conducted by galvanostatically cycling the half cell between 2.0 and 4.3 V vs. Li.

Results and Discussion

1. Al-binding Sequences via “Bio-panning” and Rational Design

The commercial phage display library Ph. D.-7 kit provided by New England Biolabs (NEB) consists of $\sim 10^9$ randomized linear heptapeptide (7-mer) at pIII sites, whose complexity is sufficient to cover all possible 7-mer sequences. When exposed to a given surface, those sequences with higher affinity would bind to the substrate and get enriched after each rinsing and amplification cycle.⁹ Typically after 4~5 rounds, all the phage particles collected by the substrate would have the identical pIII sequences. Those consensus sequences are considered to be the most efficient binding constitution toward the target surface.

Three separate parallel “bio-panning” tests were conducted on Al-foil, and two consensus sequences, named A1 and A2 respectively, were identified (Table 1).

Table 1. Consensus pIII sequences identified via “bio-panning”

Panning Number	1	2	3
pIII Sequences	STIHGST (A1)	STIHGST (A1)	ATFNTMT (A2)

Upon close examination of A1 and A2, it was found that both sequences contain high density of serine (S) and threonine (T), which leads to our suspicion that the functional group hydroxyl in S and T plays a critical role in binding these phage particles to aluminum surface. Since most metal surfaces are covered with a native oxide film, it is possible that hydroxyl interacts with the negatively-charged sites of the film in a manner similar to H-bond. To maximize this interaction, we designed two 7-mer motifs, heptaserine (S7) and heptathreonine (T7).

On the other hand, earlier studies on metal-protein interaction using yeast display libraries have also identified that positively-charged residues such as arginine (R) and lysine (K) bind strongly to metal oxide layers. While it has proven impossible to use those positively charged residues at pIII sites of M13 phage without interfering its reproduction cycle, we designed an alternative sequence heptahistidine (H7), which remains neutral at pH~7 but becomes to positively-charged at pH~5.0.

2. Genetic Engineering

Having identified the desired aluminum-binding pIII sequences, either via “bio-panning” from the pIII libraries or via “rational” designing”, we need to fuse them into a single M13 phage clone that has a pVIII sequence to bio-mineralize interested materials. Previously two useful pVIII motifs haven been reported, i.e., tetraglutamate (E4) whose excessive negative charge helps in coordinating metal cations that serve as precursor of active materials,^{4,5} and a sequence that is designed to bind to SWNT while maintaining metal-cation affinity, DVYESALP (EFE).⁶ In this work we borrowed the vectors from these M13 phage clones having E4 and EFE at pVIII, and attempted to fuse the interested pIII sequences into these vectors, as illustrated in Figure 2.

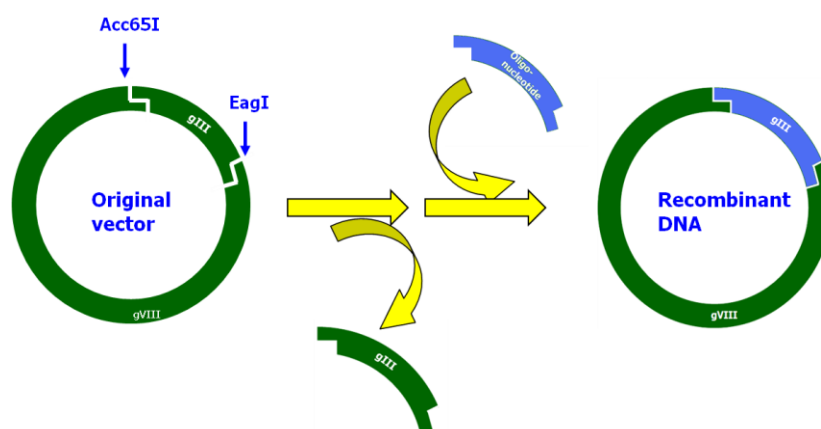


Fig. 2. Illustration of pIII cloning using vectors of E4- or EFE-bearing M13 phages.

Using established cloning protocol, vectors separated from the above M13 phages were double-digested with EagI and Acc65I. After purification and dephosphorylation,

custom-synthesized oligonucleotides encoding A1, A2, S7, T7 and H7 were fused with the vector respectively. The resultant new circular DNA will contain both a gVIII that encodes either E4 or EFE and a gIII that encodes either A1, A2, S7, T7 or H7. These DNAs were then introduced into bacteria cells for amplification. Genetic sequencing results confirmed the successful cloning (Figure 3). Seven such new clones were made as summarized in Table 2.

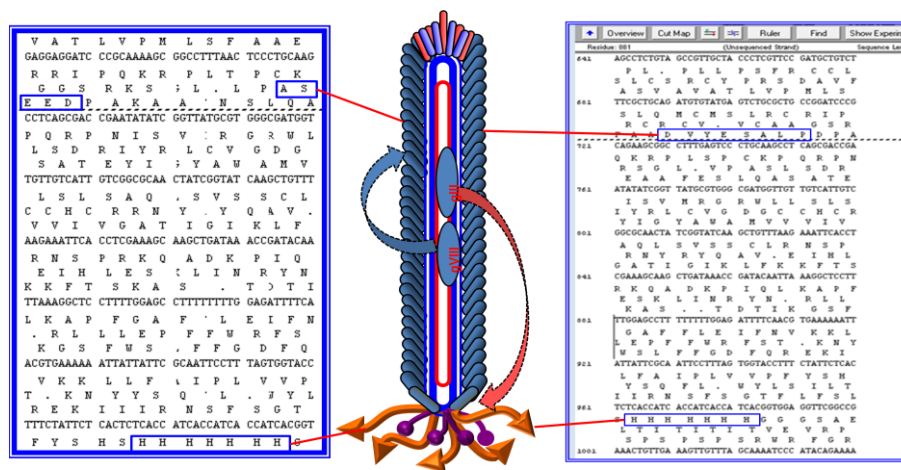


Fig. 3. Genetic sequencing results confirming the successful fusing of desired pIII and pVIII protein sequences to create new clones E4-H7 and EFE-H7.

Table 2 Recombinant M13 phages

Clone ID	pIII	pVIII
E4-A1	STIHGST	EEEE
E4-A2	ATFNTMT	EEEE
E4-S7	SSSSSSS	EEEE
E4-T7	TTTTTTT	EEEE
E4-H7	HHHHHHH	EEEE
EFE-A1	STIHGST	DVYESALP
EFE-H7	HHHHHHH	DVYESALP

3. Binding Tests and Bio-mineralization

To confirm the aluminum-binding ability of the introduced pIII motifs on the recombinant phages, several binding tests were conducted.

Assuming that the native film on aluminum is mostly alumina (Al_2O_3), sapphire was chosen as target, and incubated in E4-A1 for 2 hours. After normal washing and elution protocol, the target was imaged with atomic force microscope (AFM) as shown in Figure 4a.

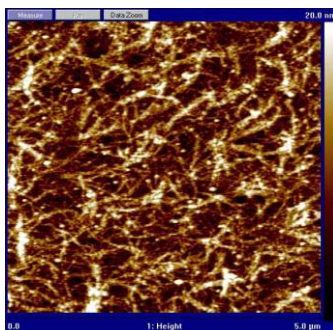


Fig. 4a. AFM images of W-A1 clone on sapphire (Al_2O_3) surface after washing and elution at low pH.

Apparently the phages indeed bind to the oxide surface with the randomized orientations. It is possible that, while the exposed pIII acts as the active binding sites, the flexible phages “pile up” on each other and forms layered deposits on the target surface. Aside from visual image, more quantitative tests were conducted by directly binding various phages on aluminum foil that is usually used as battery cathode substrate, and then eluting under strong acidic conditions (pH ~ 3.3). The eluent was then analyzed by UV-Vis spectra for phage particle concentration. The binding efficiency was evaluated by UV-Vis using an empirical equation at 1 mm oath-length:

$$\text{Phage particles/mL} = \frac{(A_{269} - A_{320})}{7225} \times 6 \times 10^{17}$$

where A stands for absorption at given wave-length.

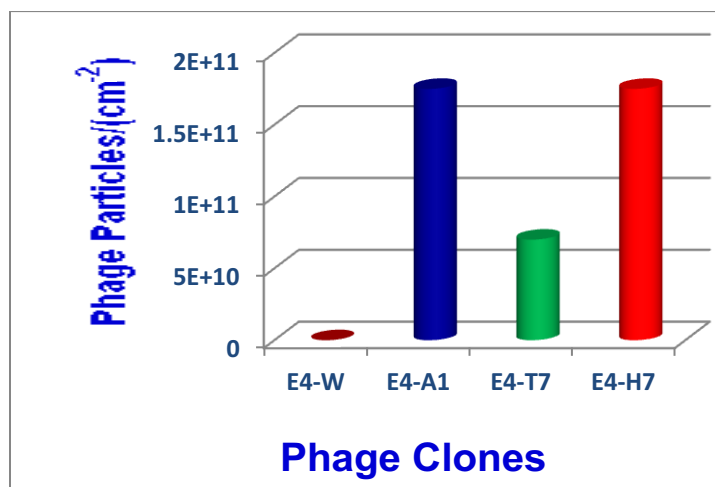


Fig. 4b. Binding experiments performed on several engineered M13 viruses on aluminum surface.

Figure 4b compared the binding strength of a few recombinant phage clones made in this work after an aluminum disc was incubated in 2×10^{12} phage particles. As reference for comparison, the E4 clone without pIII engineering (E4-W) was also used. All recombinant clones studied showed higher binding strength, with A1 and H7 motifs

being the most effective; to our surprise, however, the rational-designed motifs S7 and T7 did not provide expected enhancement in aluminum affinity.

Environmental scanning electron microscope (ESEM) graphs were taken of the aluminum surface after EFE-H7 was immobilized onto it followed by incubation with SWNT dispersion in sodium cholate (SC). Since the phage themselves cannot be imaged by SEM, SWNT are the only species that can be visualized, but knowing that the motif in the major coat protein pVIII, EFE, binds with SWNT, we can still infer from the ESEM image how the phage particles are arranged on the aluminum surface. As Figure 4c showed, the SWNT were randomly piled upon each other, forming a 3D network similar to that shown by AFM micrograph (Figure 4a). This network will be used later for self-assembly of active cathode species.

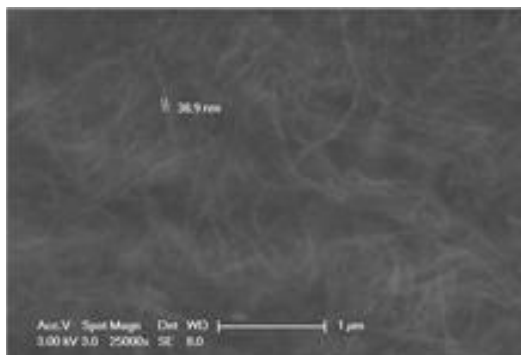


Fig. 4c. ESEM image of engineered clone EFE-H7 on aluminum after incubating with SWNT.

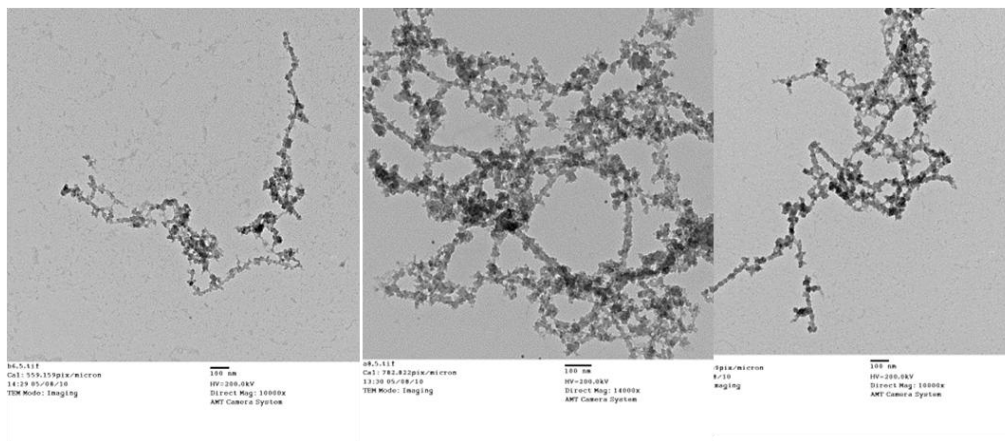


Fig. 5. TEM images of a-FePO₄-nanowires grown on engineered clone EFE-H7.

Finally, in order to confirm that the newly fused pIII does not interfere with the capability of pVIII in bio-mineralization, a-FePO₄ nanowires were grown onto E4-H7 and EFE-H7 clones using the same bio-mineralization protocol developed by Lee et al. Figures 5 showed nanowires thus obtained, usually ~1.0 μm in length and 10~20 nm in diameter, in good agreement with the report. EDX analysis confirmed the chemical composition of these nanowires as a-FePO₄.

4. *In situ* Bio-mineralization and Electrochemical Characterization

EFE-A1 and EFE-H7 clones were immobilized onto aluminum discs by incubating the latter with a concentrated TBS suspension of the former (2×10^{10} particles/ μL) in a sterilized multi-welled container. After two hours shaking and subsequent rinsing with TBS, dispersion of 2% SWNT in SC and solutions of AgOAC solution, FeCl_3 and Na_3PO_4 were added to the well according to the above sequence, with each addition separated by repeated TBS rinsings. The corresponding incubation durations were 2 hours, 48 hours, respectively.

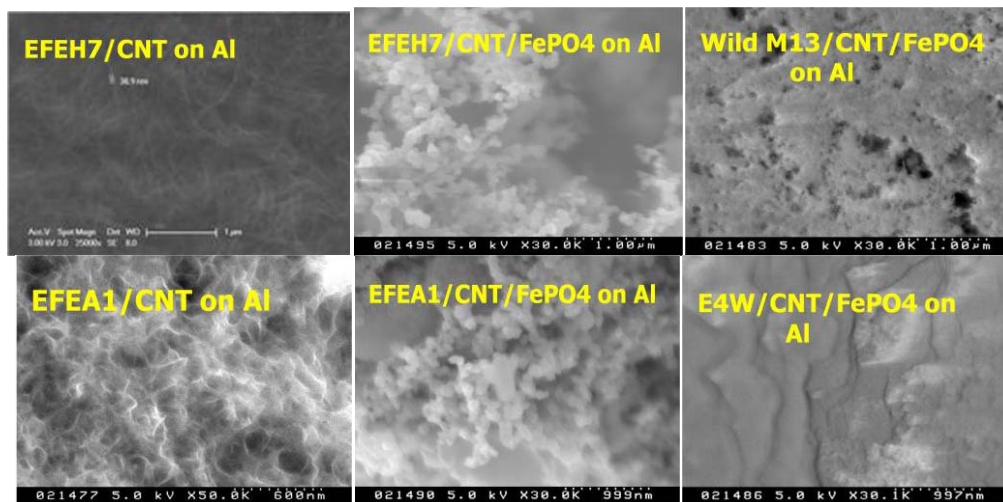


Fig. 6. ESEM and SEM images of various engineered clones on aluminum after incubating with SWNT and bio-mineralization.

The final electrodes were dried at $100\text{ }^{\circ}\text{C}$ under vacuum overnight before being assembled into coin cells with lithium as counter electrode. To provide a reference for comparison, wild-type M13 phages without any engineering or E4-W where pIII was not engineered were also used in the *in situ* bio-mineralization. Scanning electron microscope (SEM) micrographs of the electrodes thus prepared are shown in panels of Figure 6.

Apparently with both wild-type and E4-W phages the electrode surface were covered with bulk inorganic deposits, in which no nano-structure is discernible, while with both EFE-A1 and EFE-H7 clones, large portions of the electrodes consist of particles whose diameter seems to fall in a rather narrow range of 10–30 nm. The formation of the nano-structures is obviously correlated to the presence of phages on aluminum surface, which is in turn made possible by their aluminum-binding pIII motifs. Closer examination of the SEM micrographs reveals that those nano-particles seem to be “strung” together by an invisible wire, like a string of beads on a necklace. We believe that the phages are the invisible wires here judged from the particle diameter and the wire-length as observed previously in bulk bio-mineralization. Figure 7 schematically describes such a composite 3D-network assembled by this bio-template based on the above micro-images of AFM, ESEM, SEM and TEM obtained at different stages of the bio-mineralization (Figures 4, 5 and 6).

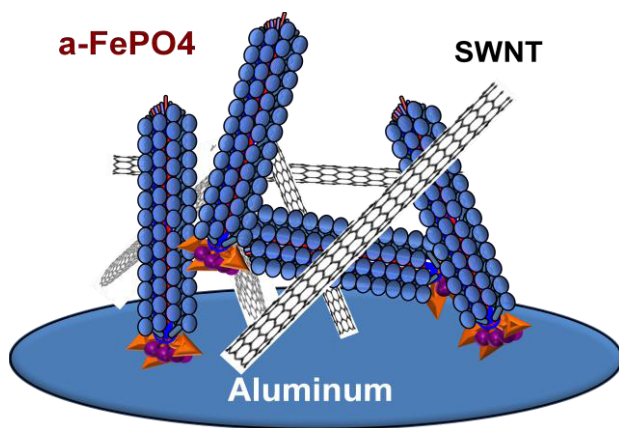


Fig. 7. Schematic illustration of an electrochemically active 3D network assembled by the virus template on aluminum surface.

Electrochemical characterization of these in-situ bio-mineralized electrodes covered with self-assembled monolayers of α -FePO₄ was conducted by galvanostatic cycling at $1 \mu\text{A}/\text{cm}^2$ between 2~4.3 V. In case of wild-type and E4-W clones, large irreversible capacities were observed in the initial cycles, while those clones with A1 and H7 all show varying degrees of electrochemical activity and reversibility in the range between 2.8~3.5 V vs. Li, typical of the lithiation/de-lithiation of α -FePO₄. In the presence of SWNT, which serves as conductive pathways, the utilization of α -FePO₄ seems to be unaffected; however, the electrochemical reversibility is significantly improved when an EFE-H7 clone is used in the presence of various SWNT concentrations (Figure 8a and 8b).

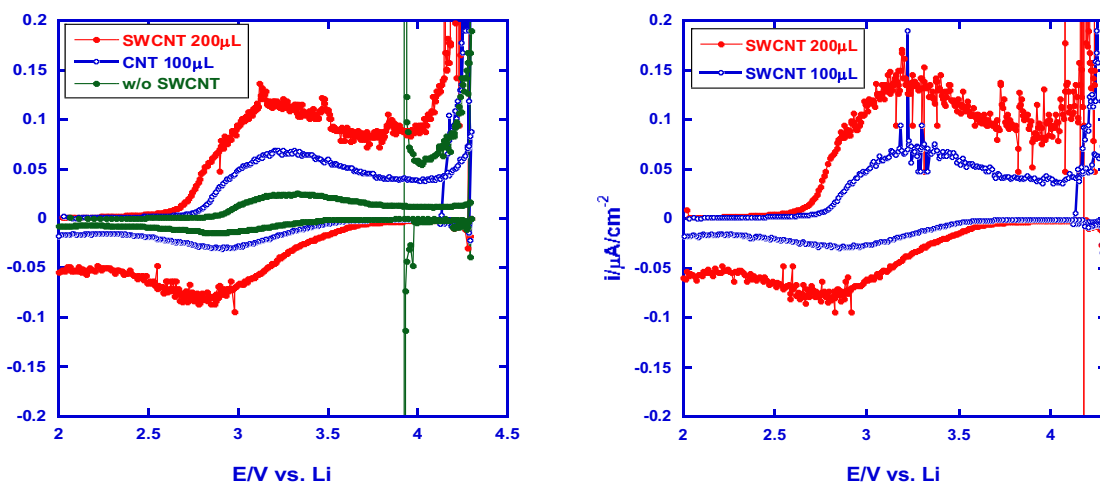


Fig. 8a. Differential capacity of α -FePO₄/CNT composite layer assembled by EFE-H7 on aluminum. (a, left): the effect of SWNT; (b, right): the reproduction of electrochemical activity with identical cells.

These preliminary characterizations demonstrated that the 3D-composite networks assembled by the genetically engineered M13 phages on Al-foil are indeed electrochemically active species. Considering that, in the absence of any conductive

carbon usually used in battery electrode building, the only conductive pathway between the active species ($\alpha\text{-FePO}_4$) and current collector ($\text{Al}_2\text{O}_3/\text{Al}$) is the SWNT, the electrochemical activity observed is very encouraging.

While this work only aims at demonstrating the feasibility of tailoring the virus structures for material functions, we firmly believe that the potential of this emerging technique are not limited to battery applications only; instead it provides a universally powerful tool to wire materials at nano-scale. In future work we will pursue to exploit this technique for other applications and devices.

Conclusions

By reprogramming the genetic codes of M13 bacteriophages, we were able to manipulate their coat protein expressions, which interface active materials, conductive pathway and current collector surface and weave them into an electrochemically active 3D composite network.

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